

LISTING OF CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims

1. **(Currently Amended)** A reaction mixture for primer-based amplification and detection of a target nucleic acid, the reaction mixture comprising:
each conventional nucleotide dATP, dCTP, and dGTP, and a combination of unconjugated dUTP and dTTP in an amount equivalent to the concentrations of dATP, dCTP and dGTP, wherein said unconjugated dUTP is at a concentration of about 10% to about 100% of the concentration-of said dTTP in said combination; and at least one of a fluorescent probe, beacon or intercalating dye;

wherein the inclusion of unconjugated dUTP reduces the formation of primer aggregates during the amplification reaction in comparison with an amplification reaction employing only conventional nucleotides; and

wherein said reaction mixture lacks a uracil degradation enzyme.

2. **(Currently amended)** The reaction mixture according to claim 1, wherein the unconjugated dUTP replaces from about 10 to about 30% of the dTTP in said reaction mixture.

3. **(Currently amended)** The reaction mixture according to claim 1, wherein the unconjugated dUTP replaces from about 20 to about 40% of the dTTP in said reaction mixture.

4. **(Currently amended)** The reaction mixture according to claim 1, further comprising at least one additional unconventional nucleotide, wherein the combined concentration of said unconjugated dUTP and said at least one unconventional nucleotide does not exceed 75% of any one conventional nucleotide in said reaction mixture.

5. **(Previously Presented)** The reaction mixture according to claim 1, wherein said reaction mixture comprises a primer pair and wherein each member of the primer pair has at least one or more uracil bases incorporated therein.

6. **(Original)** The reaction mixture according to claim 5, wherein each member of the primer pair has all of its thymidine bases replaced with uracil bases.

7. **(Currently amended)** The reaction mixture according to claim 1, wherein the unconjugated dUTP does not exceed a final amplification reaction concentration of about 300 μM .

8. **(Currently amended)** The reaction mixture according to claim 1, wherein the unconjugated dUTP does not exceed a final amplification reaction concentration of about 100 μ M.

9. **(Previously Presented)** The reaction mixture according to claim 1, further comprising at least one polymerase enzyme.

10. **(Previously Presented)** The reaction mixture according to claim 1, further comprising a buffer system.

11. **(Currently Amended)** A method for reducing primer aggregation during amplification and detection of target nucleic acid, the method comprising:

combining a target nucleic acid with a reaction mixture comprising each conventional nucleotide dATP, dCTP, and dGTP and a combination of dTTP with unconjugated dUTP in an amount equivalent to the concentrations of dATP, dCTP and dGTP; wherein said unconjugated dUTP is at a concentration of about 10% to about 100% of the concentration of said dTTP in said combination;

amplifying the target nucleic acid to produce amplicons; and

detecting the amplicons so produced;

wherein the level of primer aggregate formed during the amplification step is reduced as compared to amplifying the target nucleic acid using a dNTP mix having only conventional nucleotides, wherein said method lacks an enzyme degradation step to degrade uracil-containing amplicons, and wherein said nucleic acid is DNA.

12. **(Canceled)**

13. **(Previously Presented)** The method according to claim 11, wherein the reaction mixture further comprises sorbitol or mannitol.

14. **(Original)** The method according to claim 13, wherein the target nucleic acid has secondary structure.

15-18. **(Canceled)**

19. **(Previously Presented)** The method according to claim 13, wherein the reaction mixture comprises 100 to 500 mM sorbitol or 100 to 300 mM mannitol .

20. **(Previously Presented)** The reaction mixture according to claim 1, wherein the reaction mixture further comprises sorbitol or mannitol.

21. **(Previously presented)** The reaction mixture according to claim 20, wherein the reaction mixture comprises 100 to 500 mM sorbitol or 100 to 200 mM mannitol.

22.-23. **(Cancelled)**

24. **(Currently amended)** The method according to claim 11, wherein the unconjugated dUTP replaces from about 10 to about 30% of the dTTP in said reaction mixture.

25. **(Currently amended)** The method according to claim 11, wherein the unconjugated dUTP replaces from about 20 to about 40% of the dTTP in said reaction mixture.

26. **(Currently amended)** The method according to claim 11, further comprising at least one additional unconventional nucleotide, wherein the combined concentration of said unconjugated dUTP and said at least one unconventional nucleotide does not exceed 75% of any one conventional nucleotide in said reaction mixture.

27. **(Currently amended)** The method according to claim 11, wherein the unconjugated dUTP does not exceed a final amplification reaction concentration of about 300 μ M.

28. **(Currently amended)** The method according to claim 11, wherein the unconjugated dUTP does not exceed a final amplification concentration of about 100 μ M.

29. **(Previously Presented)** The method according to claim 11, further comprising at least one polymerase enzyme.

30. **(Previously Presented)** The method according to claim 11, further comprising a buffer system.

31. **(Canceled)**

32. **(Previously Presented)** The method according to claim 11, wherein said amplifying step comprises heating said target nucleic acid to a temperature greater than 60° C.